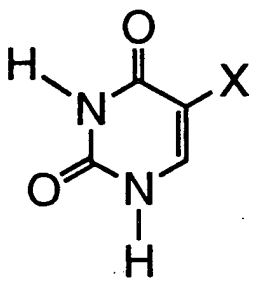
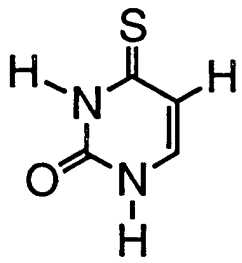
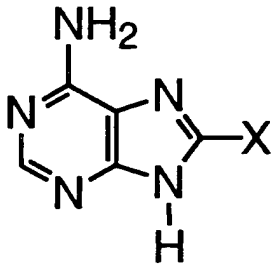


X=Br, 5-bromouracil
X=I, 5-iodouracil
X=N_3, 5-azidouracil



4-thiouracil



X=N_3, 8-azidoadenine
X=Br, 8-bromoadenine

FIG. 1

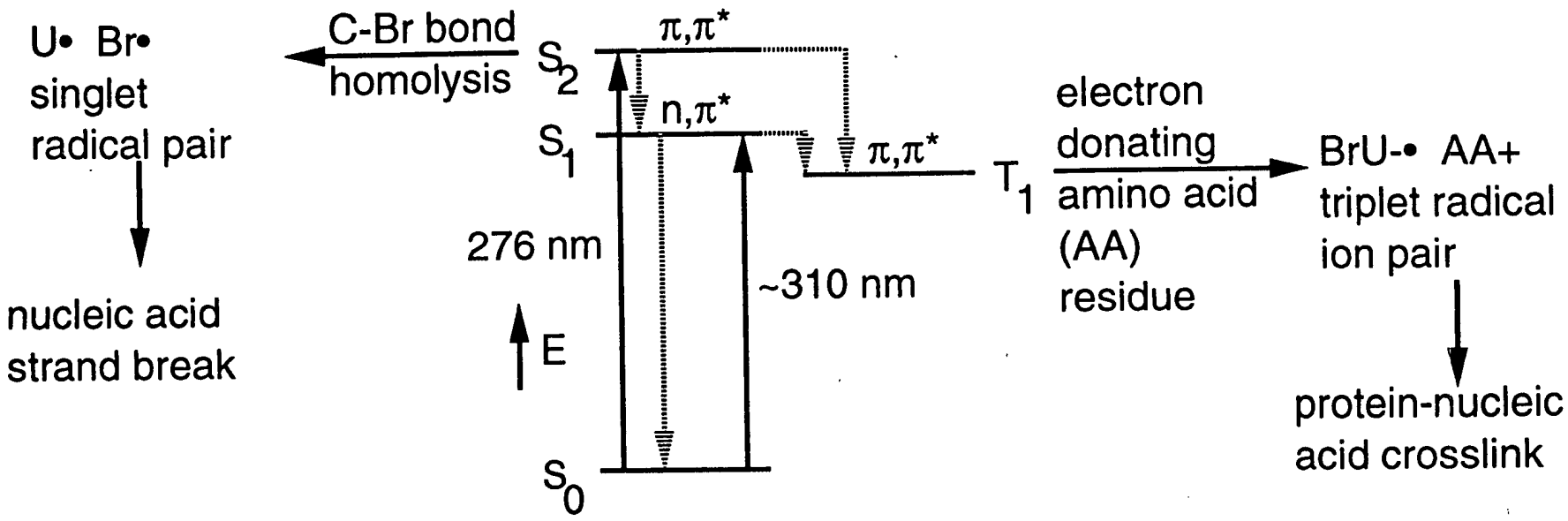

U• Br•
singlet
radical pair
nucleic acid
strand break
C-Br bond
homolysis
S2
S1
S0
π,π*
n,π*
π,π*
T1
276 nm
E
~310 nm
electron
donating
amino acid
(AA)
residue
BrU-• AA+
triplet radical
ion pair
protein-nucleic
acid crosslink

FIG. 2

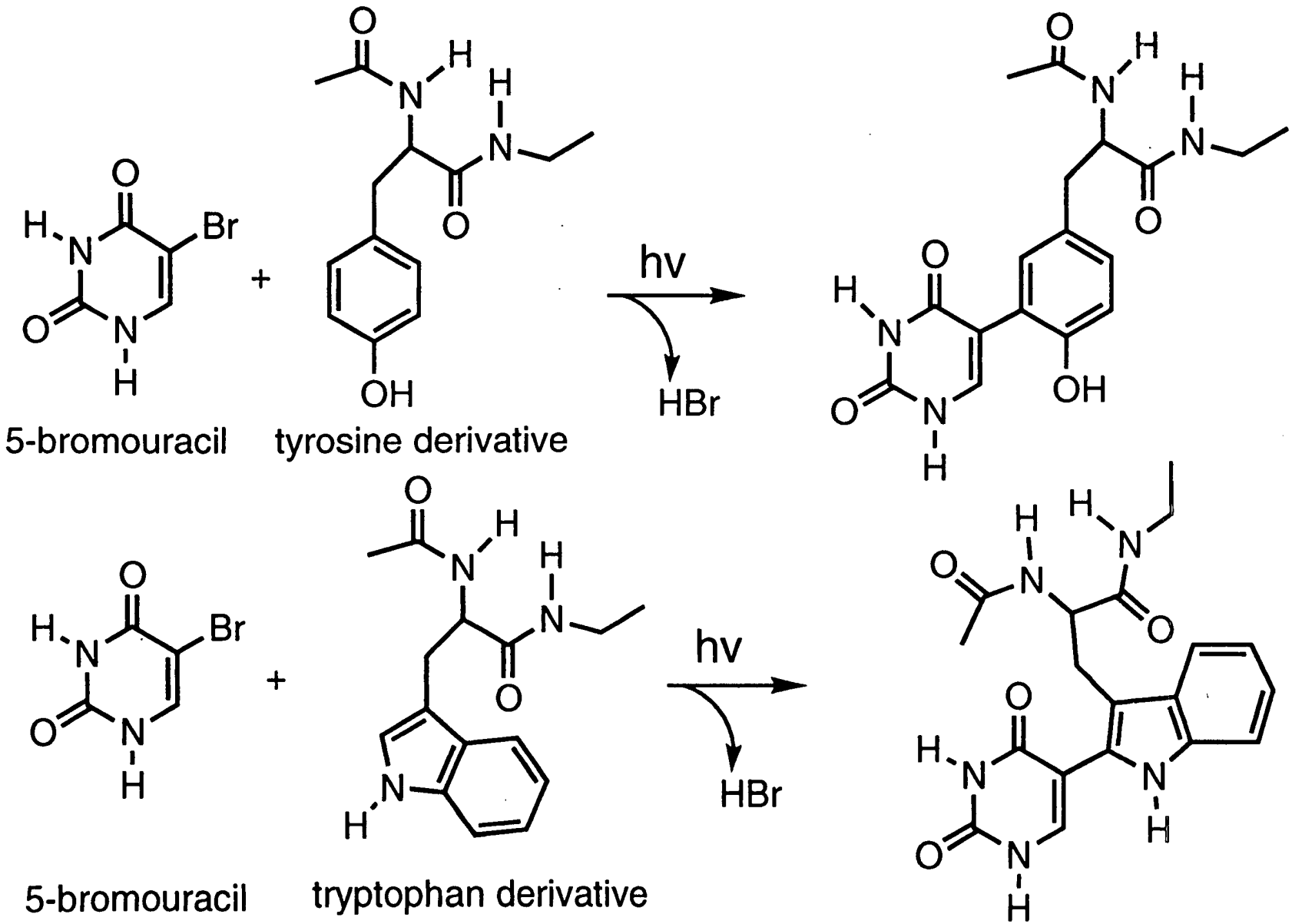

5-bromouracil
tyrosine derivative
hv
HBr
5-bromouracil
tryptophan derivative
hv
HBr

FIG. 3

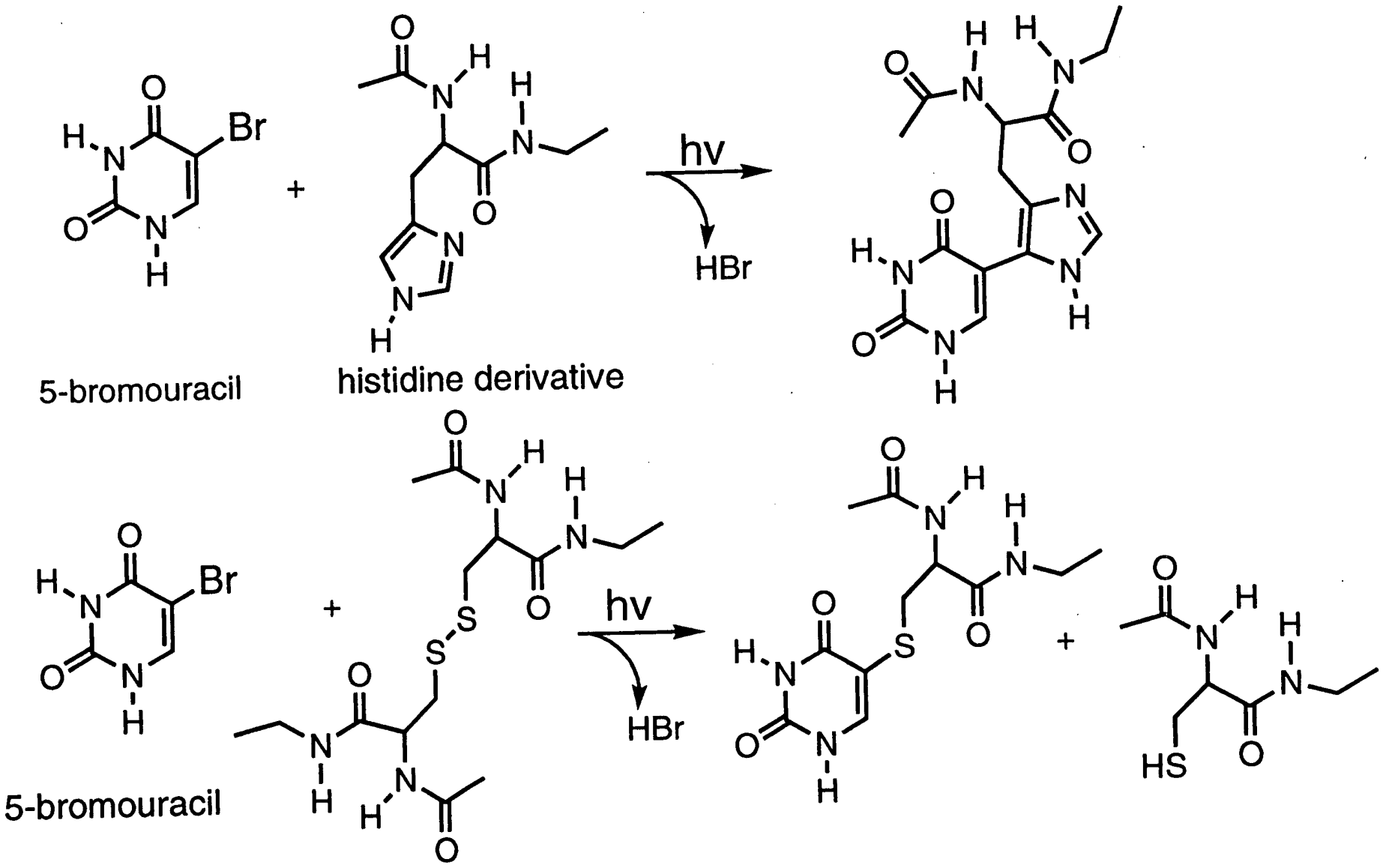

5-bromouracil
histidine derivative
hv
HBr
5-bromouracil
cystine derivative
hv
HBr

FIG. 3 (CONT'D)

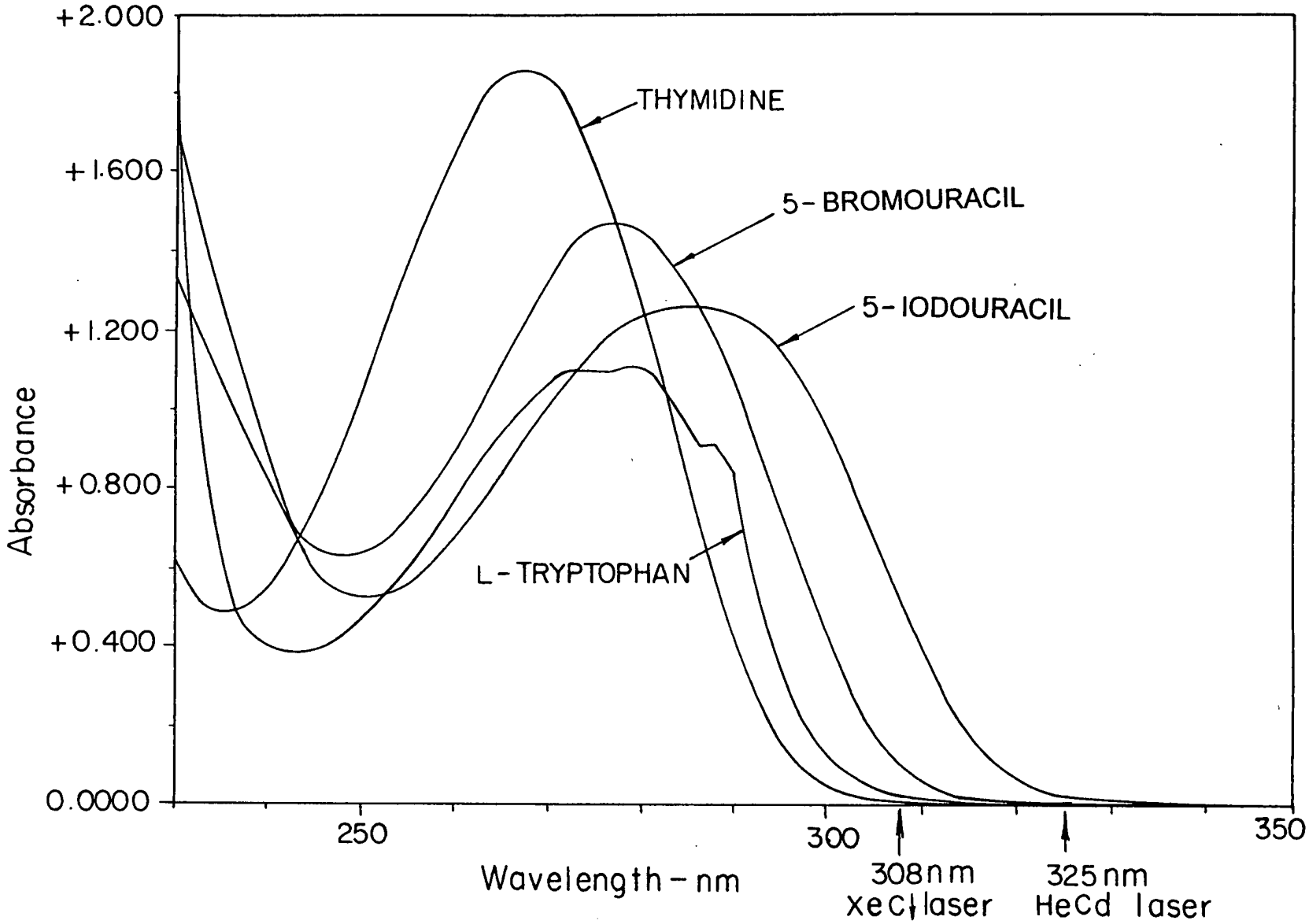
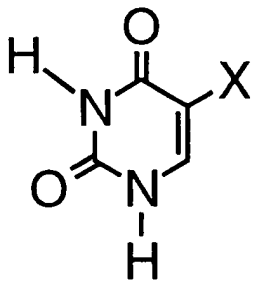
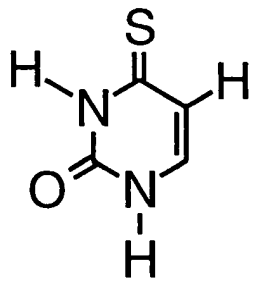
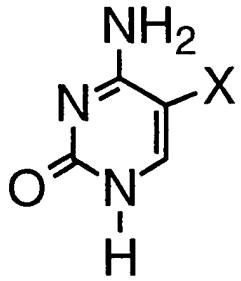

+2.000
+1.600
+1.200
+0.800
+0.400
0.0000
Absorbance
THYMIDINE
5-BROMOURACIL
5-IODOURACIL
L-TRYPTOPHAN
250
300
350
Wavelength-nm
308nm
XeCl laser
325nm
HeCd laser

FIG.4





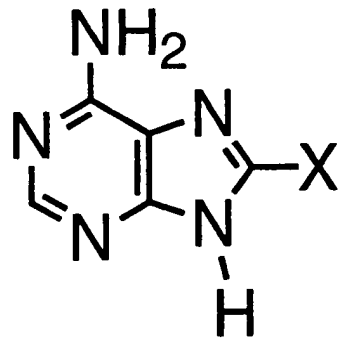
4-thiouracil



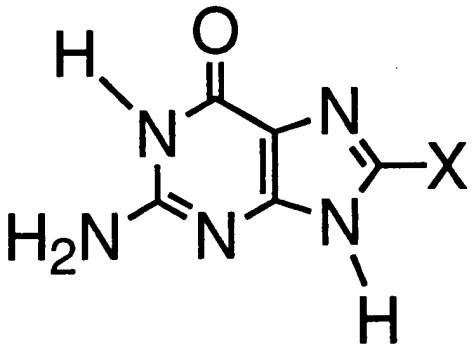
X=Br, 5-bromouracil
X=I, 5-iodouracil
X=CH_2=CHBr, 5-bromovinyluracil
X=CH_2=CHI, 5-iodovinyluracil
X=N_3, 5-azidouracil

X=Br, 5-bromocytosine
X=I, 5-iodocytosine
X=CH_2=CHBr, 5-bromovinylcytosine
X=CH_2=CHI, 5-iodovinylcytosine
X=N_3, 5-azidocytosine

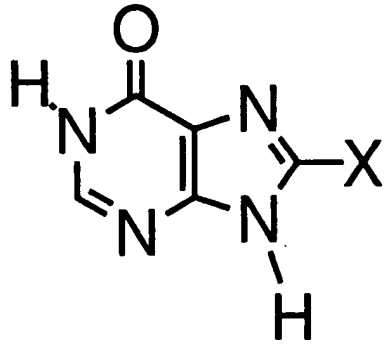
FIG. 5



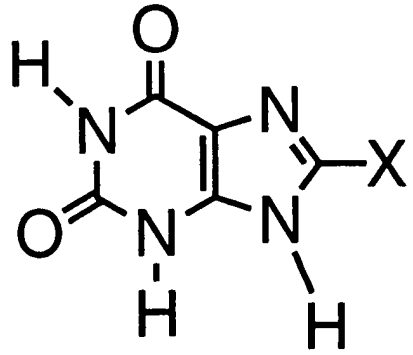
X=N_3, 8-azidoadenine
X=Br, 8-bromoadenine
X=I, 8-iodoadenine



X=N_3, 8-azidoguanine
X=Br, 8-bromoguanine
X=I, 8-iodoguanine

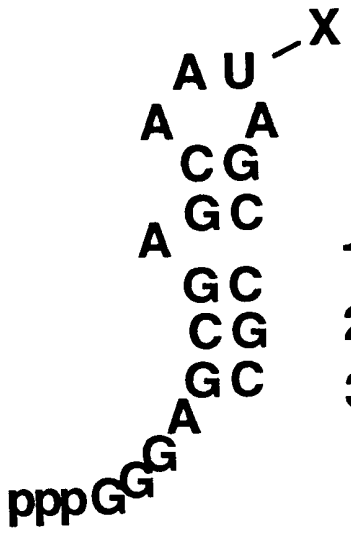


X=N_3, 8-azidohypoxanthine
X=Br, 8-bromohypoxanthine
X=I, 8-iodohypoxanthine



X=N_3, 8-azidoxanthine
X=Br, 8-bromoxanthine
X=I, 8-iodoxanthine

FIG. 5 (CONT'D)



1, X = Br (SEQ ID NO:1)

2, X = I (SEQ ID NO:2)

3, X = H (SEQ ID NO:3)

FIG. 6

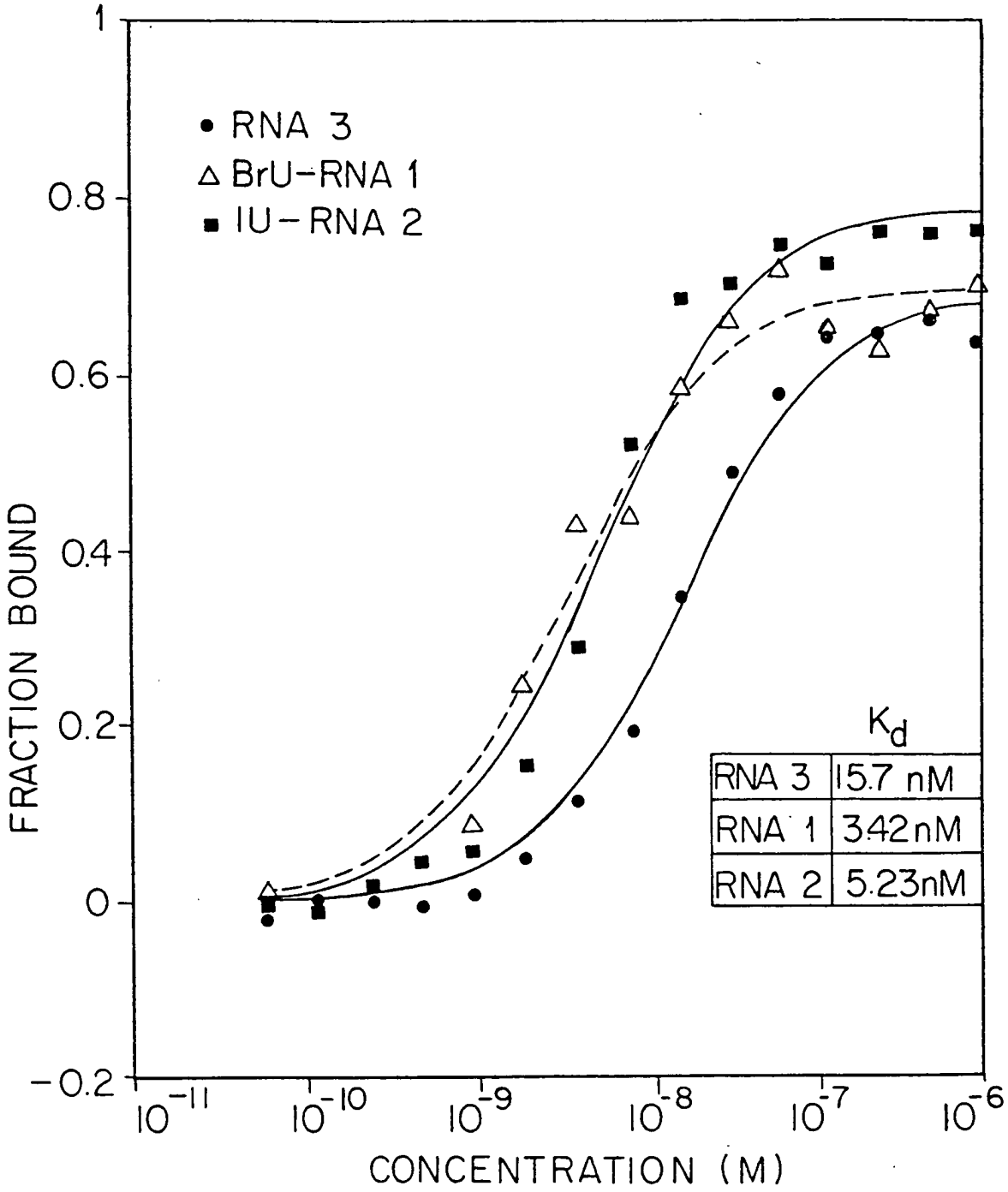

1
0.8
0.6
0.4
0.2
0
-0.2
FRACTION BOUND
• RNA 3
△ BrU-RNA 1
■ IU-RNA 2
K_d
RNA 3 | 15.7 nM
RNA 1 | 3.42nM
RNA 2 | 5.23nM
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
CONCENTRATION (M)

FIG. 7

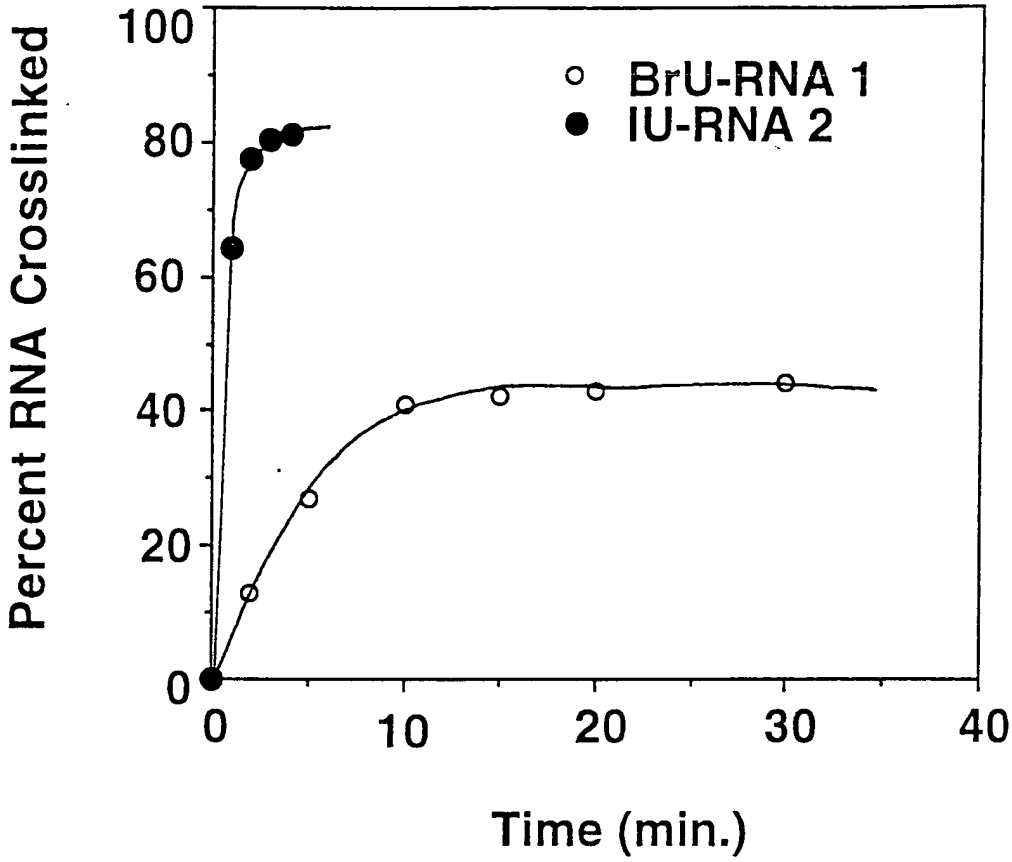

Percent RNA Crosslinked
100
80
60
40
20
0
0
10
20
30
40
Time (min.)
BrU-RNA 1
IU-RNA 2

FIG. 8

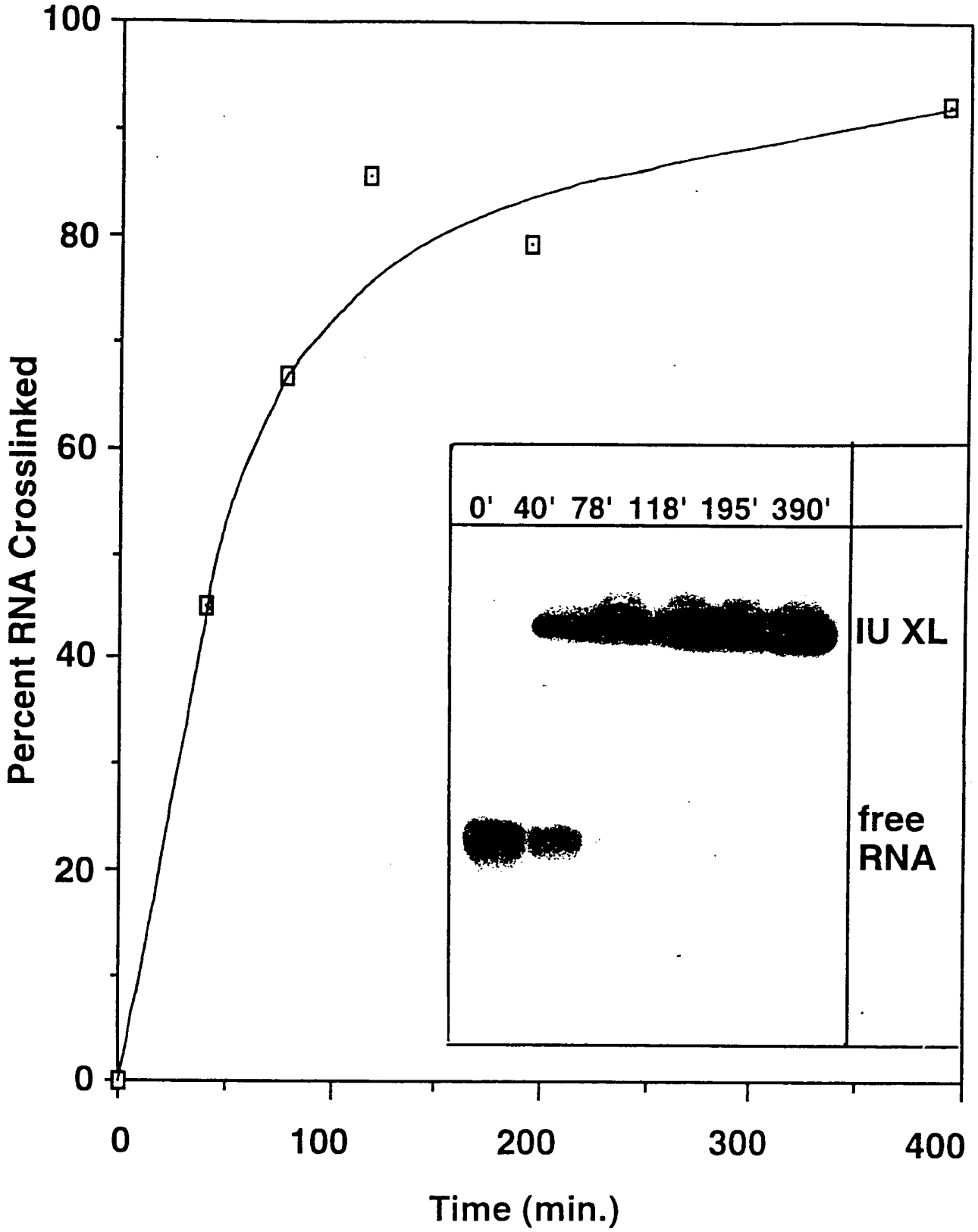

100
80
60
40
20
0
Percent RNA Crosslinked
0
100
200
300
400
Time (min.)
0' 40' 78' 118' 195' 390'
IU XL
free
RNA

FIG. 9

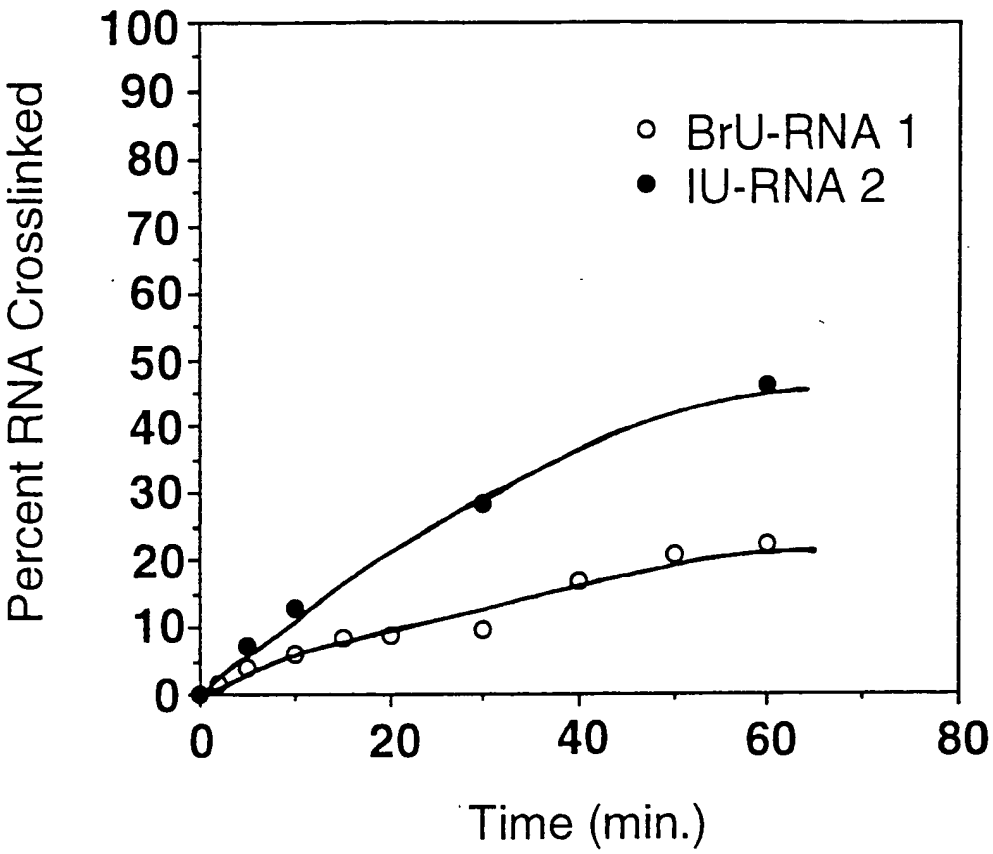

Percent RNA Crosslinked
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
Time (min.)
BrU-RNA 1
IU-RNA 2

FIG. 10

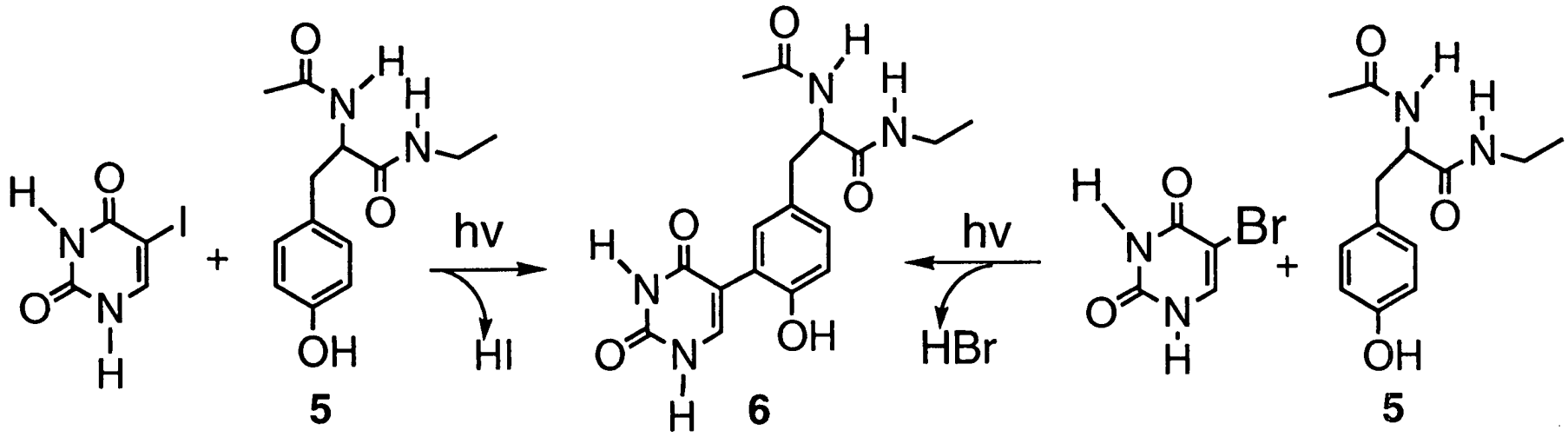


Fig. 11

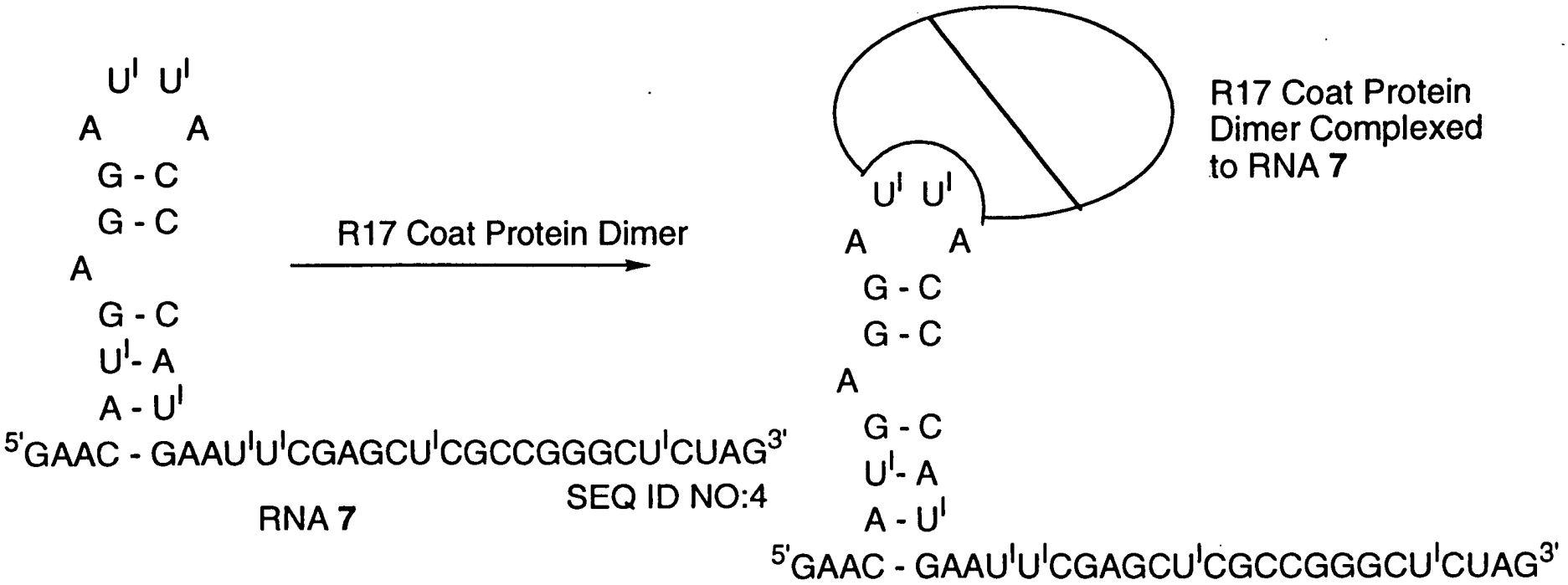

U' U'
A A
G - C
G - C
A
G - C
U'- A
A - U'
5'GAAC - GAAU'U'CGAGCU'CGCCGGGCU'CUAG3'
RNA 7
R17 Coat Protein Dimer
SEQ ID NO:4
R17 Coat Protein Dimer Complexed to RNA 7
U' U'
A A
G - C
G - C
A
G - C
U'- A
A - U'
5'GAAC - GAAU'U'CGAGCU'CGCCGGGCU'CUAG3'

FIG. 12

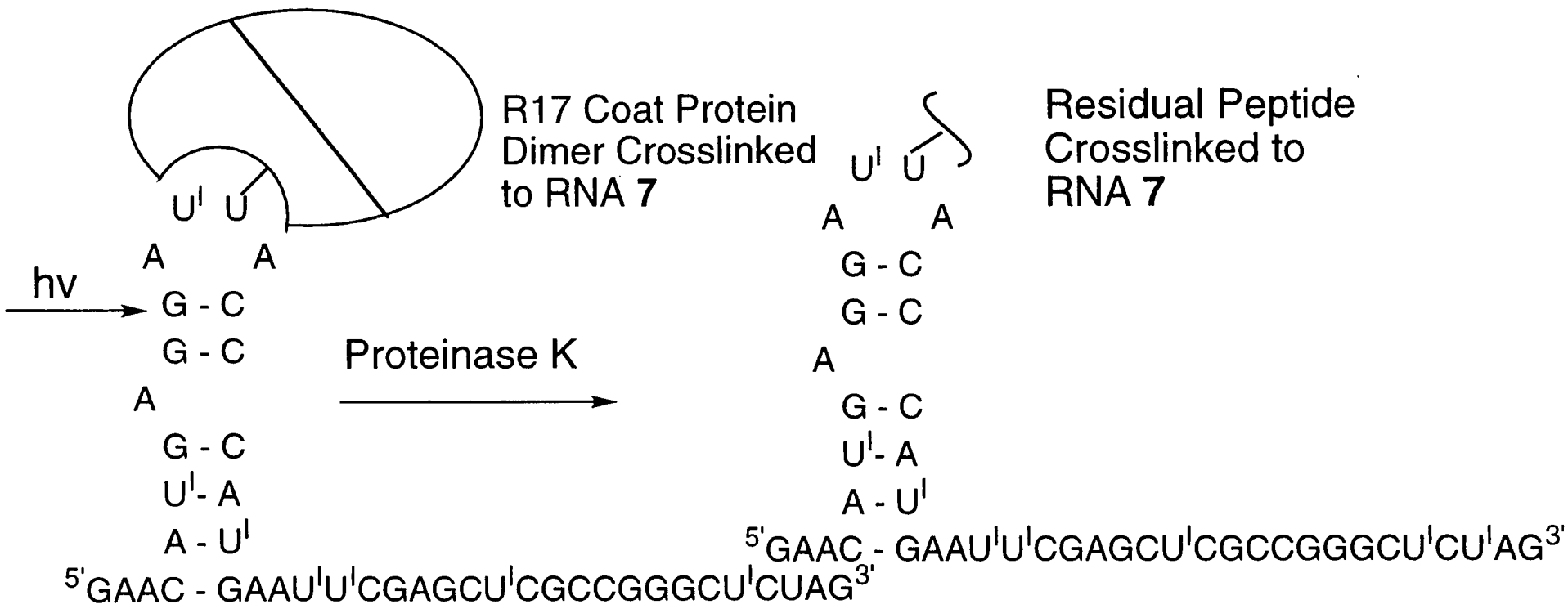

R17 Coat Protein
Dimer Crosslinked
to RNA 7
Residual Peptide
Crosslinked to
RNA 7
hv
Proteinase K
5'GAAC - GAAUIUICGAGCUICGCCGGGCUICUIAG3'
5'GAAC - GAAUIUICGAGCUICGCCGGGCUICUAG3'

FIG. 12 (CONT'D)

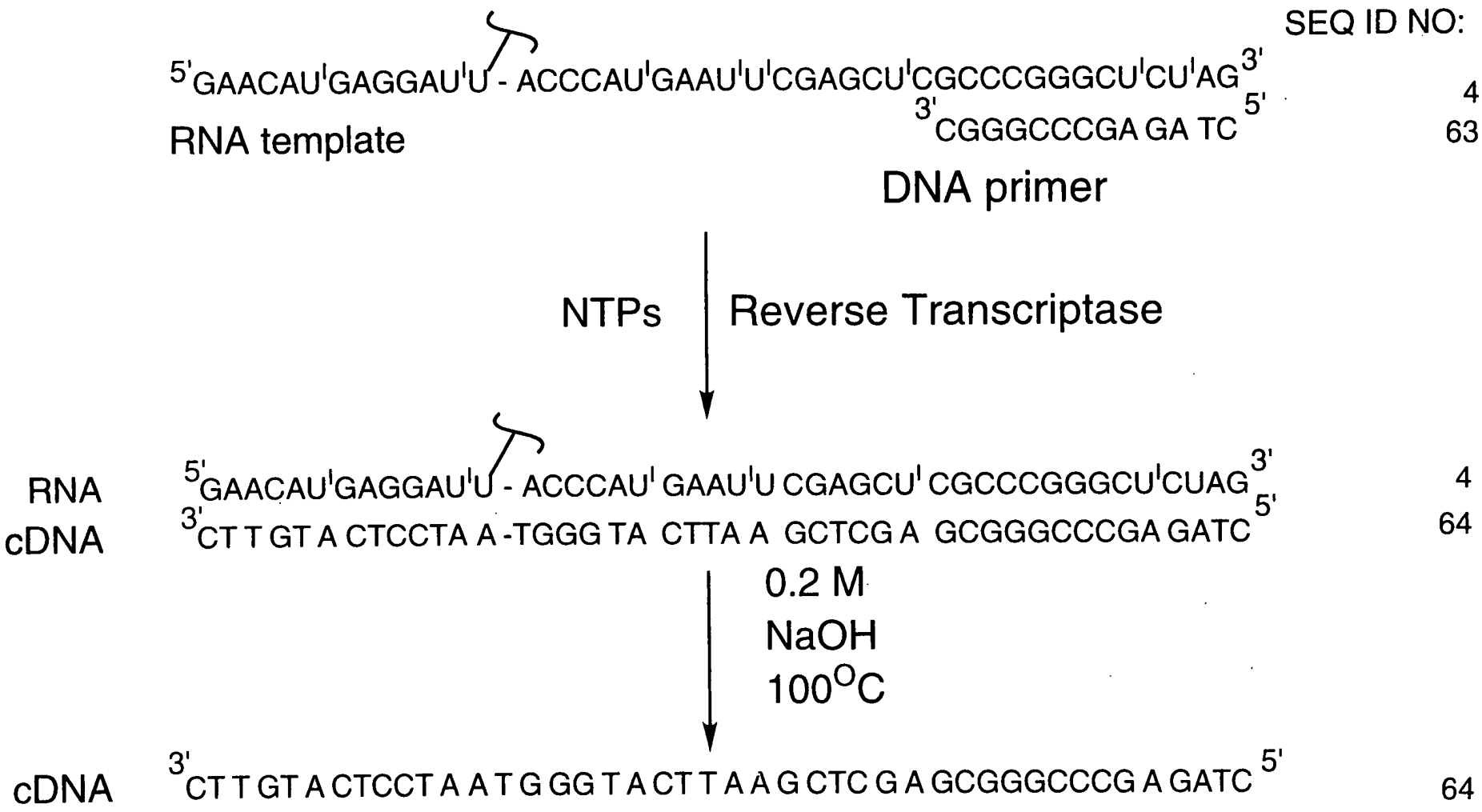

SEQ ID NO:
5'GAACAUIGAGGAUIU - ACCCAUIGAAUIUICGAGCUICGCCCGGGCUICUIAG3'
4
3'CGGGCCCGA GA TC5'
63
RNA template
DNA primer
NTPs
Reverse Transcriptase
RNA
5'GAACAUIGAGGAUIU - ACCCAUI GAAUIU CGAGCUI CGCCCGGGCUICUAG3'
4
cDNA
3'CT T GT A CTCCTA A -TGGG TA CTTA A GCTCG A GCGGGCCCGA GATC5'
64
0.2 M
NaOH
100°C
cDNA
3'CT T GT A CTCCT A A T G GG T A CT T A A G CTC G A GCGGGCCCG A GATC5'
64

FIG. 13

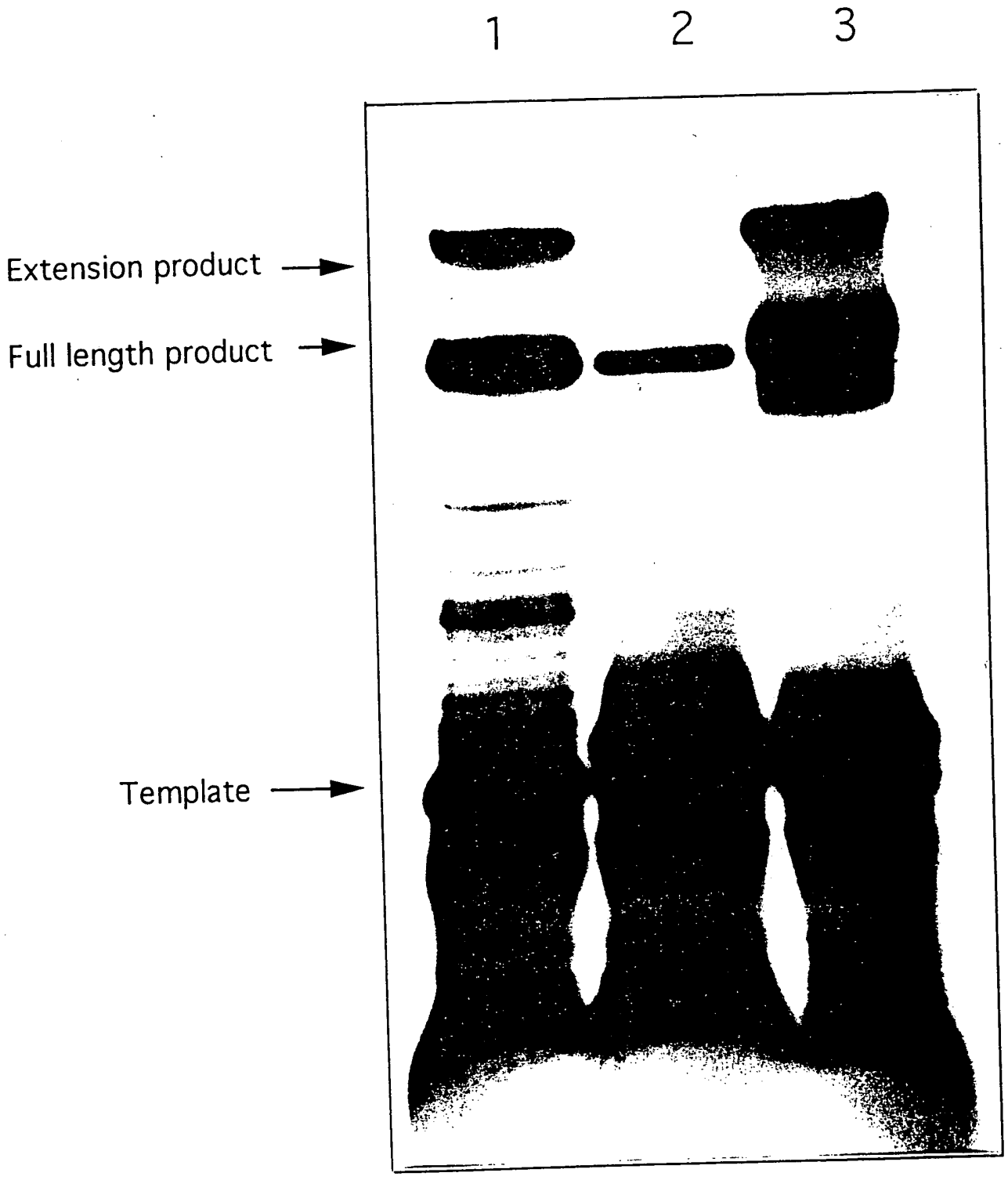


FIG. 14

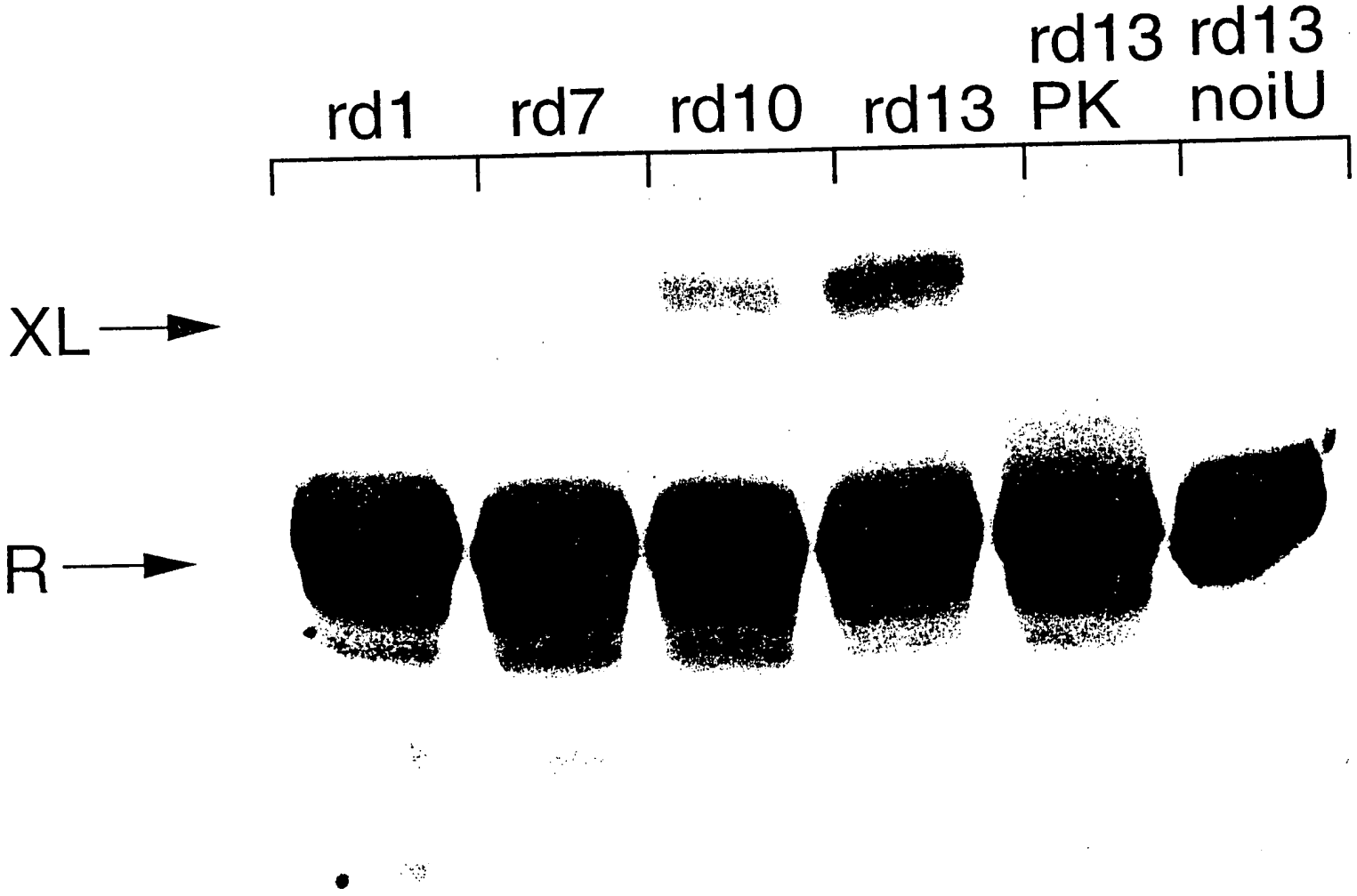


FIG. 15

			SEQ ID NO:
6a	gggugcauugagaaaacacguuuguggacucuguaucu		5
class 1		**LI-XL**	
a : 49	agguacgauuAACAg--acgacUGUUaacggccuaccu		6
65	uaacggcuuAACAagcaccauUGUUaaccuagugccu		7
11	gaguggcuuAACAagcaccauUGUUaaccuaguaccu		8
25	gugcagauuAACAacaac-guUGUUaacuccuccucu		9
*24	cuguggauuAACAggcacaccUGUUaaccguguaccu	+	10
38	cuguggauuAACAggcacaccUGUUaaccguguaccc		11
58	agacgauuAACAuccacggaUGUUaacgcgcuagaa		12
6	aagacgauuAACAaacacguuUGUUaacgcaacaccu	-	13
66	gauuggauuAACAggcaccccUGUUaa-ccuaccacu	+	14
45	aggaggauuAACAacaaaggguUGUUaaccccguacca		15
40	ugaaggauuAACAacuaauguUGUUaaccaugua	-	16
52	uugaggauuAACAggcacaccUGcUaaccguguaccc		17
27	auguggcuuAACAaguacgcuUGUUaacccaaaaacg		18
20	aggacgaugAACAaacacguuUGUUcacgccaugc		19
41	gacuggcuuAACAaacauguuuUGUUaaccguguacca	+	20
33	cggcggauuAACAcgacacacucgUGUUaaccauauc		21

FIG. 16

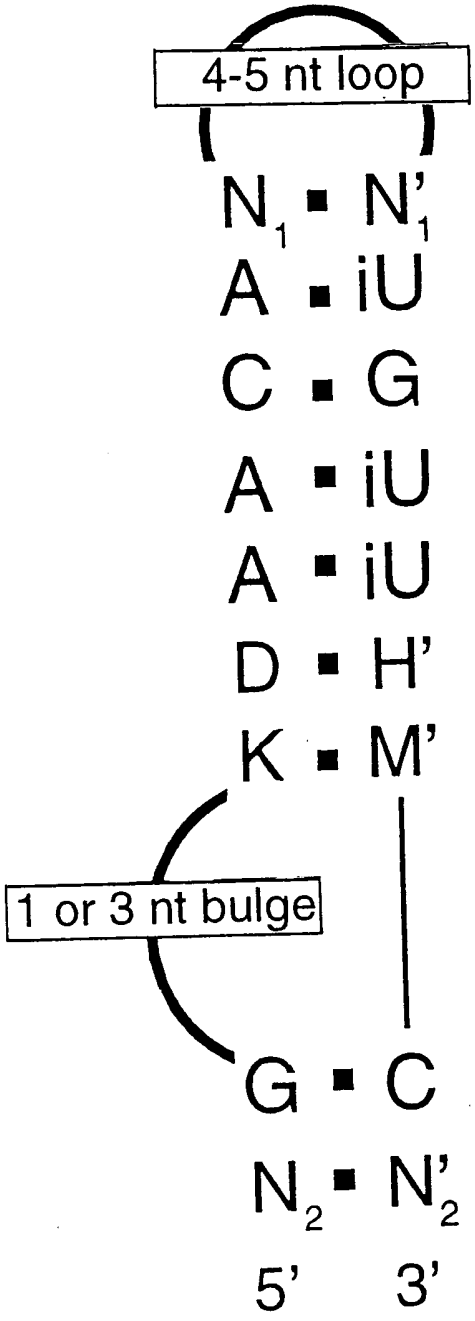
				SEQ ID NO:
b :	17	gcaucagaugAACAgcacgucUGUUcacuaugcaccc	-	22
	57	gcaucagaugAACAgcacgucUGUUcacuaugcaccu		23
	42	gcaucagauggACAgcacgucUGUUcacuaugcaccu		24
	3	caguguaugaAACAccacgugUGUUuccacuguaccu		25
	37	caguguaugaAACAacacguuUGUUuccacugccu		26
	4	gaguguaugaAACAacacguuUGUUuccacucccu		27
	36	gaguguaugaAACAacacguuUGUUuccacugucu		28
	44	gauuguaugaAACAacguguuUGUUuccacucccu		29
	7	gaauguaugaAACAacacguuUGUUuccacugccu		30
	51	gauuggacuuAACAgacacccccUGUUaaccuaccacu		31
c :	15	ugcgacaguuagaAACAcgauUGUUuacuguaug		32
	47	uacaggcuuaagaAACAcguuUGUUaaccaaccccu		33
	14	ucgagcagugugaAACAcgauugUGUUuccugcuca		34
	62	ugaugccuagagaAACAcauuagUGUUucccucugu	-	35
	54	acgugccucuagaAACAcaucugaUGUUucccucuca		36
	56	acccgccucgugaAACAcgcuugaUGUUucccucuca		37
	48	cggugacguaugaAACAcguucguUGaUUuccgu	-	38
	2	gcuugcgaAACAcguuugacgUGUUucccu		39
	10	gcacccuagaAACgcguuaguagacGUUucccu		40

FIG. 16 (CONT'D)

				SEQ ID NO:
d :	22	aggaaccuagaAACAcacagUGUUucccucugcccac	-	41
	26	gccugcauggauuAACAcguaugUGUUaaccgacucc		42
	*18	ugaAACAcugagaaacacgUGUUucccuugugugau	-	43
class 2				
	61	aGGAACCUCAAgccgccccuagaacacucggcaccu		44
	8	aGGAACCUCAAgaaagccccugaaacacucgaagccu	-	45
	53	aGGAACCUCAAgaaaccccccugaaacacucauuaccg	-	46
	39	aGGAACCUCAAgaaauccgaacgacaacccuacaccu		47
	59	aGGAACCUCAAgaaaccccgccacggaccccaacca		48
	35	gGGAACCUCAAuaaucacgcacgcauacucggcaucu		49
	29	gGGAACCUCAAgagacccgacaggauacucggac		50
	46	aaguGGAACCUCAAucccguaagaagauccuguaccu		51
	9	augugcauagagauguacauauGGAACCUCAGuagag	-	52
	5	ucaugcauaggcauaggcagauGGAACCUCAGuagcc		53
	31	augugcaacaaggcgcacggauaaGGAACCUCGAagu	-	54
	19	gaguacagcacgcaacacguacggGGAACCUCAAagu		55

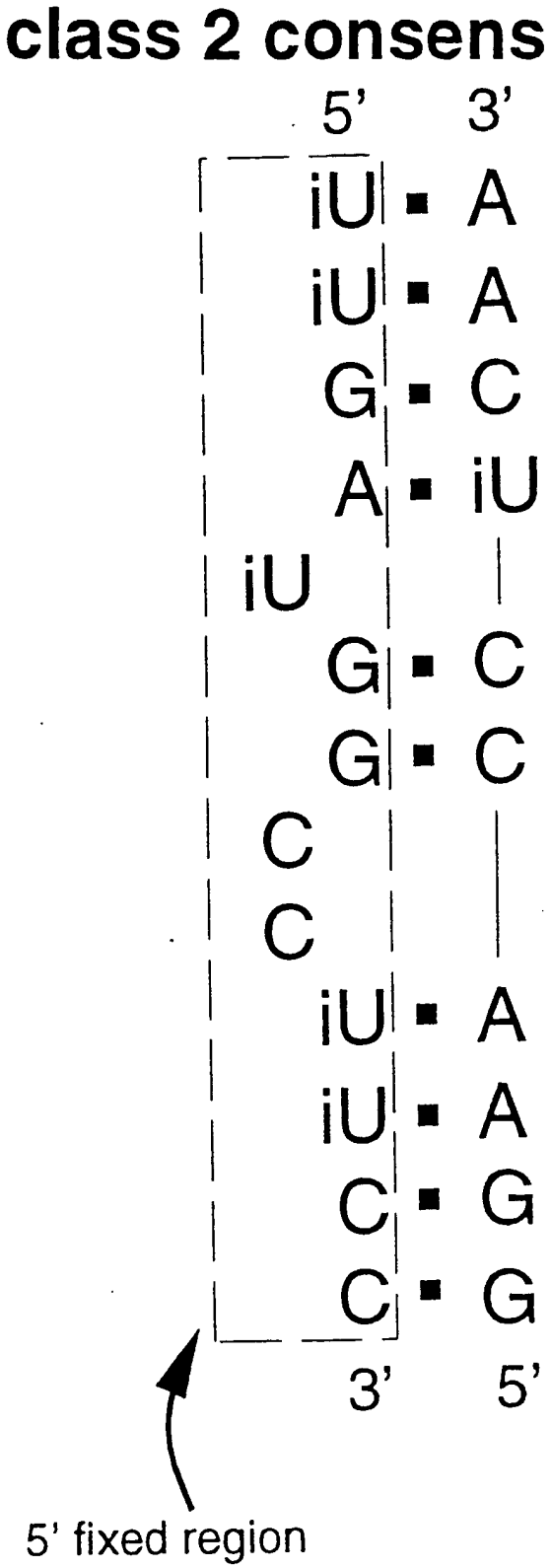
FIG. 16 (CONT'D)

class 1 consensus



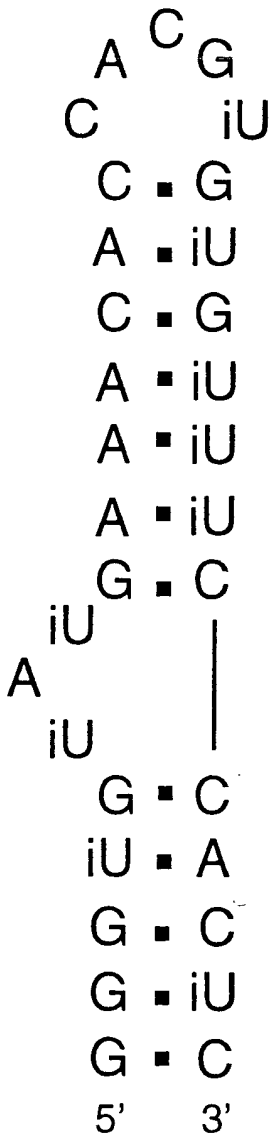
SEQ ID NO:56

FIG. 17



SEQ ID NO:57

FIG. 17 (CONT'D)



trunc2

SEQ ID NO:58

FIG. 18A

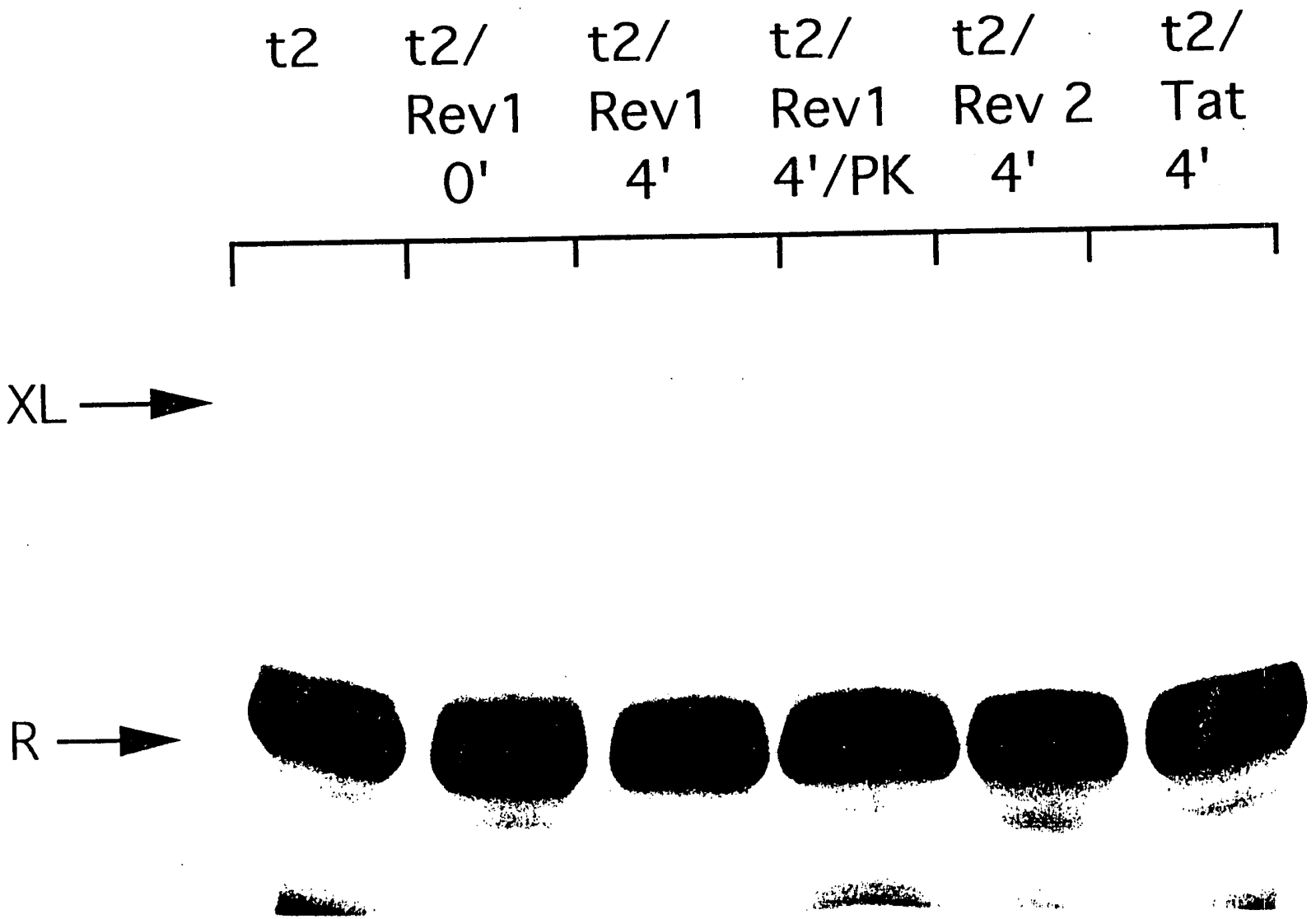
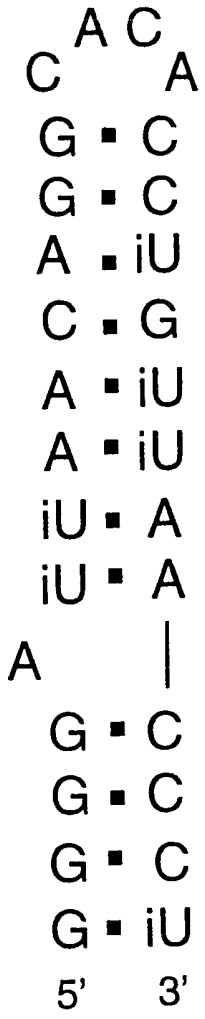


FIG. 18B



trunc24

SEQ ID NO:59

FIG. 19A

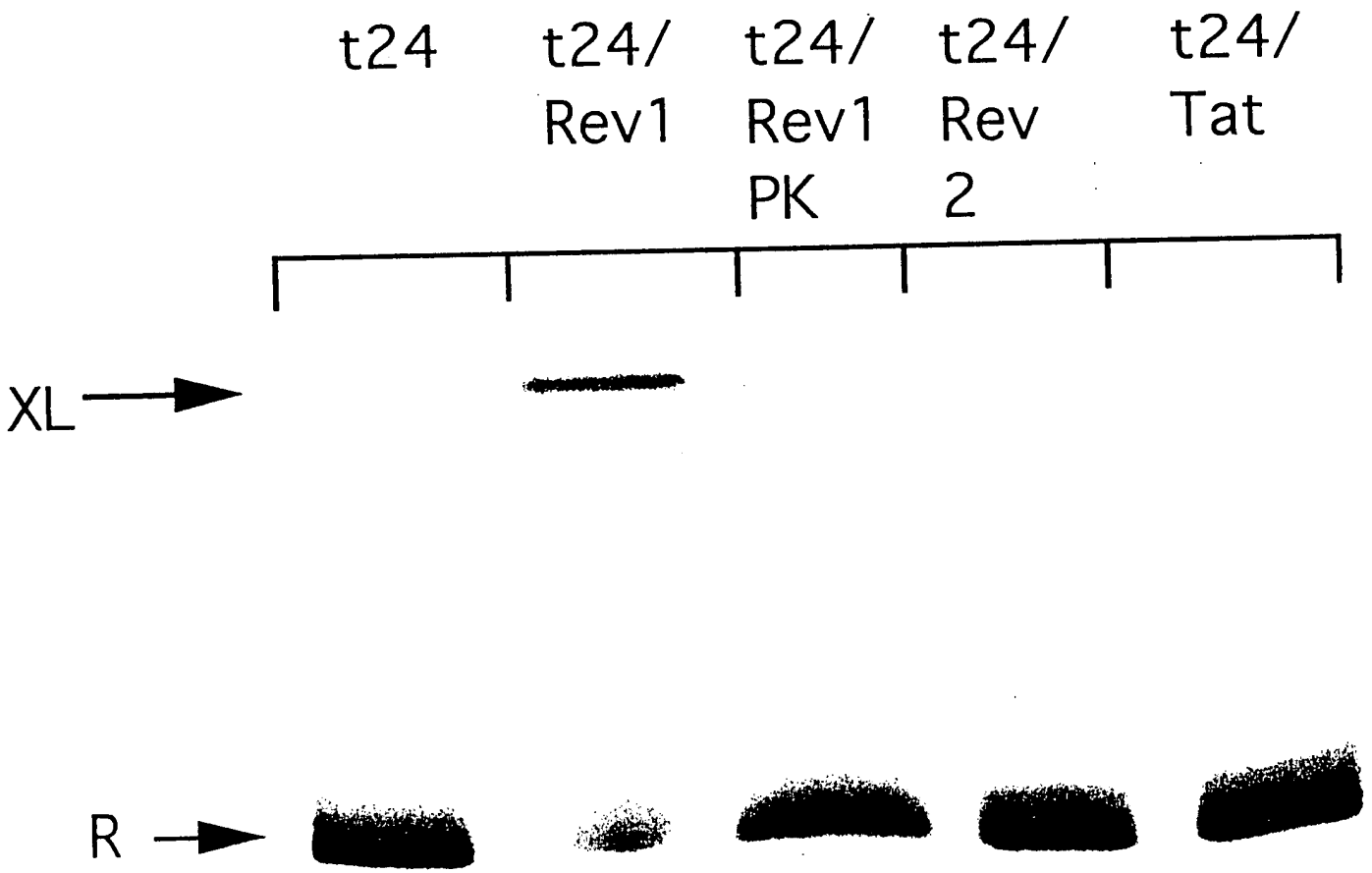


FIG. 19B

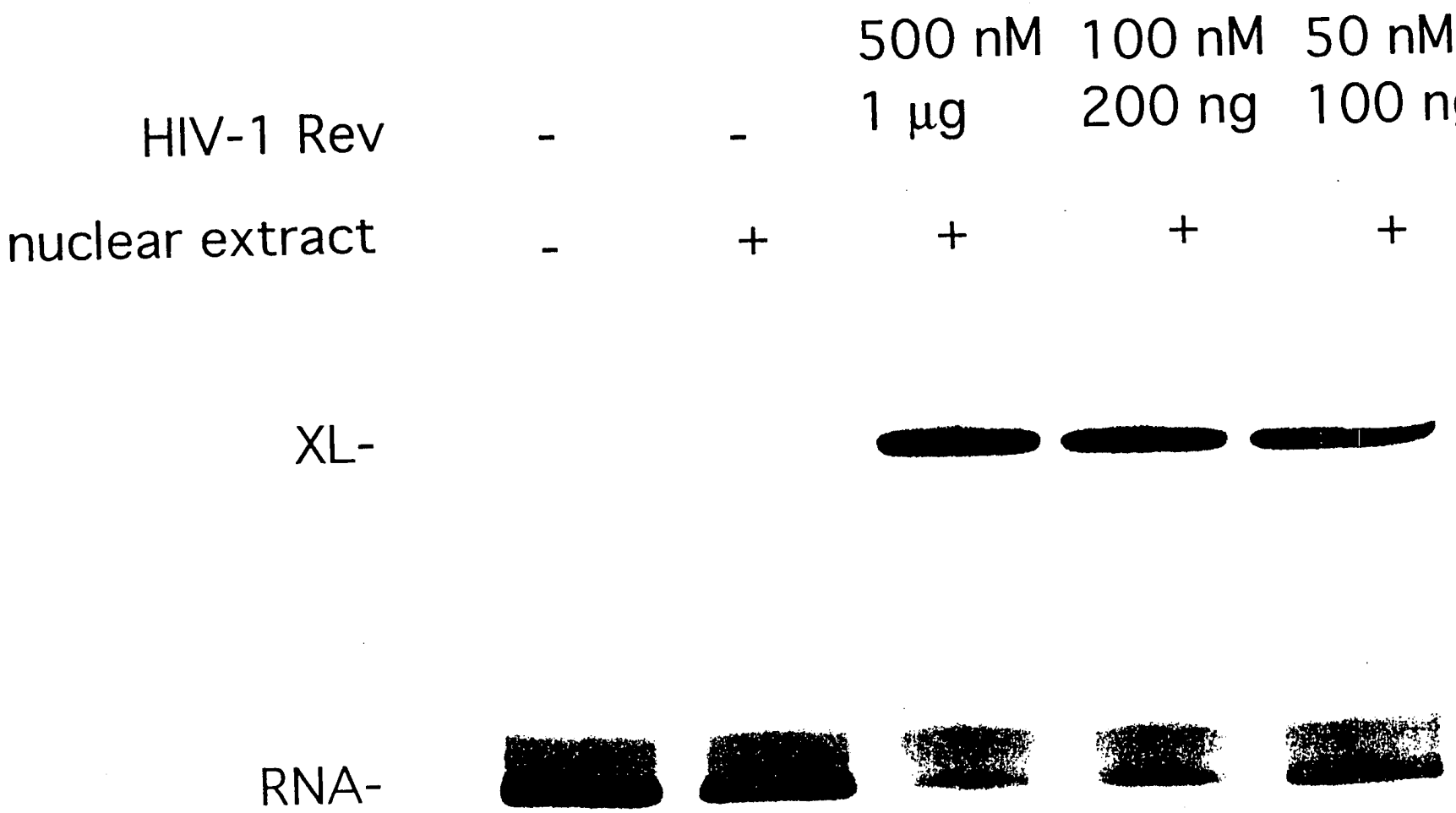

500 nM
100 nM
50 nM
1 µg
200 ng
100 ng
HIV-1 Rev
-
-
nuclear extract
-
+
+
+
+
XL-
RNA-

FIG. 20

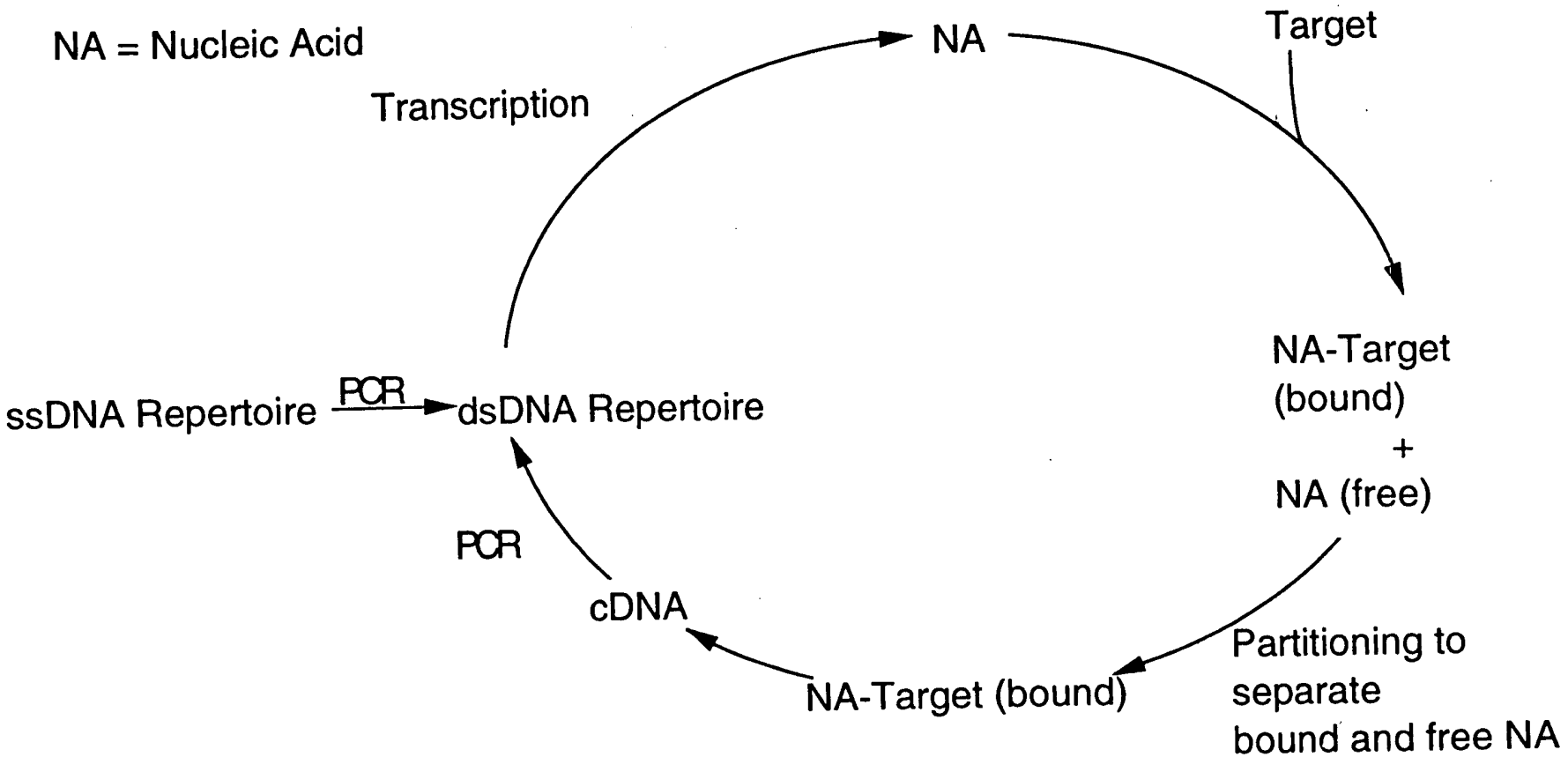

NA = Nucleic Acid
NA
Target
Transcription
NA-Target
(bound)
+
NA (free)
ssDNA Repertoire
PCR
dsDNA Repertoire
PCR
cDNA
NA-Target (bound)
Partitioning to
separate
bound and free NA

FIG. 21

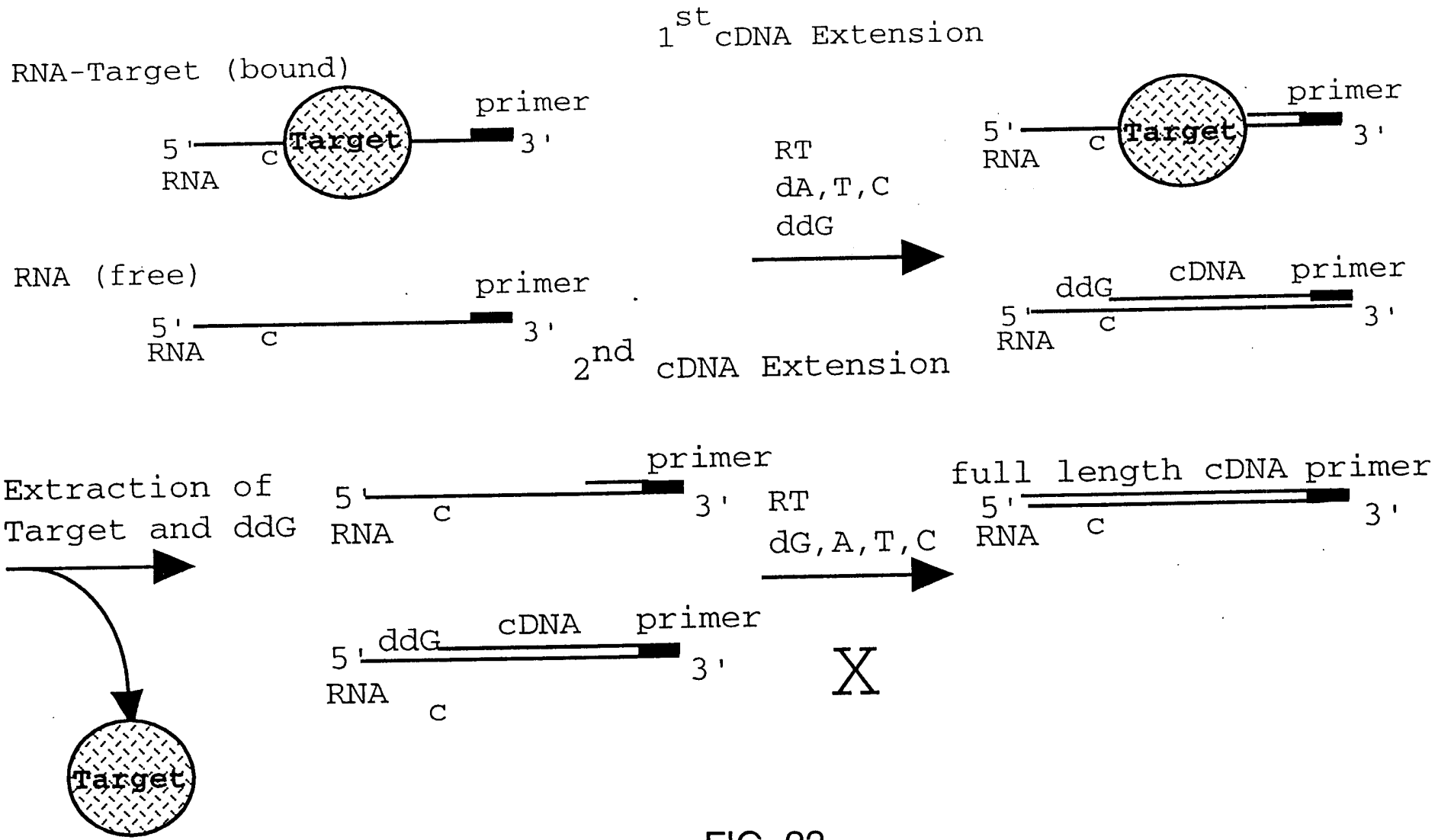

1st cDNA Extension
RNA-Target (bound)
primer
5'
RNA
c
Target
3'
RT
dA,T,C
ddG
RNA (free)
primer
5'
RNA
c
3'
ddG
cDNA
primer
2nd cDNA Extension
Extraction of
Target and ddG
primer
full length cDNA primer
RT
dG,A,T,C
ddG
cDNA
primer
X
Target

FIG. 22

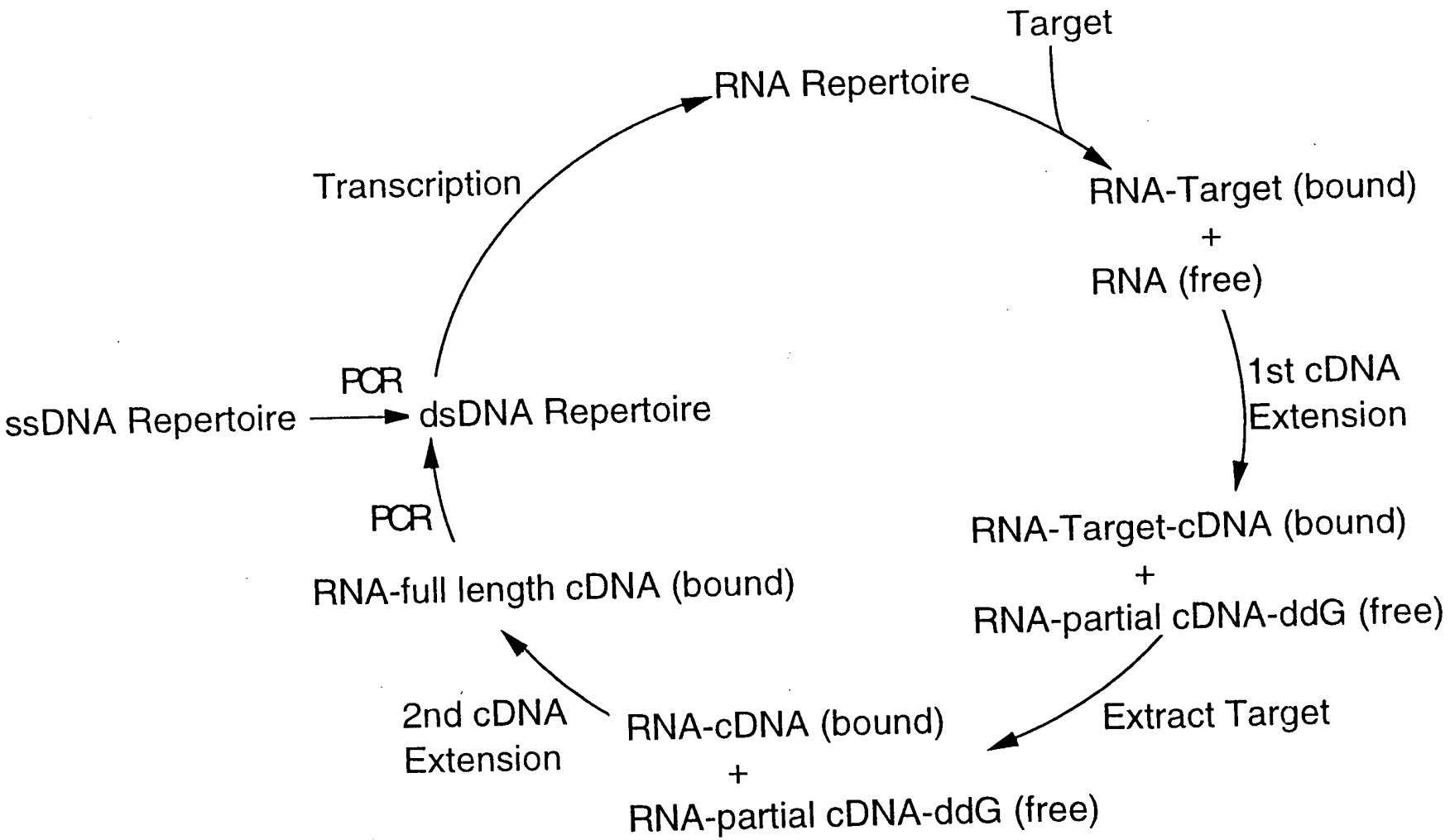

Target
RNA Repertoire
Transcription
RNA-Target (bound)
+
RNA (free)
PCR
ssDNA Repertoire
dsDNA Repertoire
1st cDNA
Extension
PCR
RNA-Target-cDNA (bound)
+
RNA-partial cDNA-ddG (free)
RNA-full length cDNA (bound)
2nd cDNA
Extension
RNA-cDNA (bound)
+
RNA-partial cDNA-ddG (free)
Extract Target

Figure 23

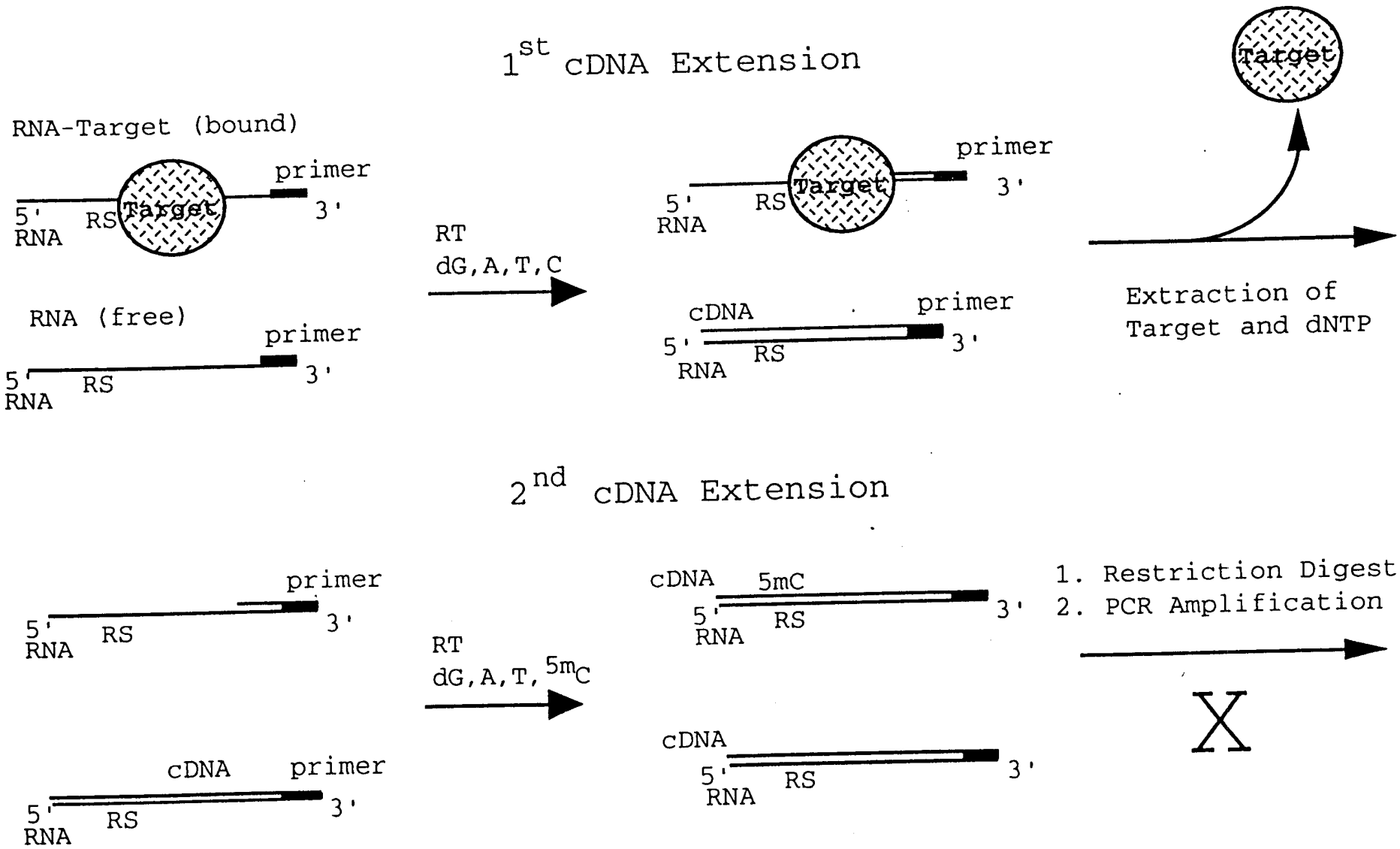

1st cDNA Extension
RNA-Target (bound)
primer
5'
RS
Target
3'
RNA
RT
dG,A,T,C
RNA (free)
primer
5'
RS
3'
RNA
cDNA
primer
5'
RS
3'
RNA
Target
Extraction of
Target and dNTP
2nd cDNA Extension
primer
5'
RS
3'
RNA
RT
dG,A,T,5mC
cDNA
primer
5'
RS
3'
RNA
cDNA
5mC
5'
RS
3'
RNA
1. Restriction Digest
2. PCR Amplification
cDNA
5'
RS
3'
RNA

Figure 24

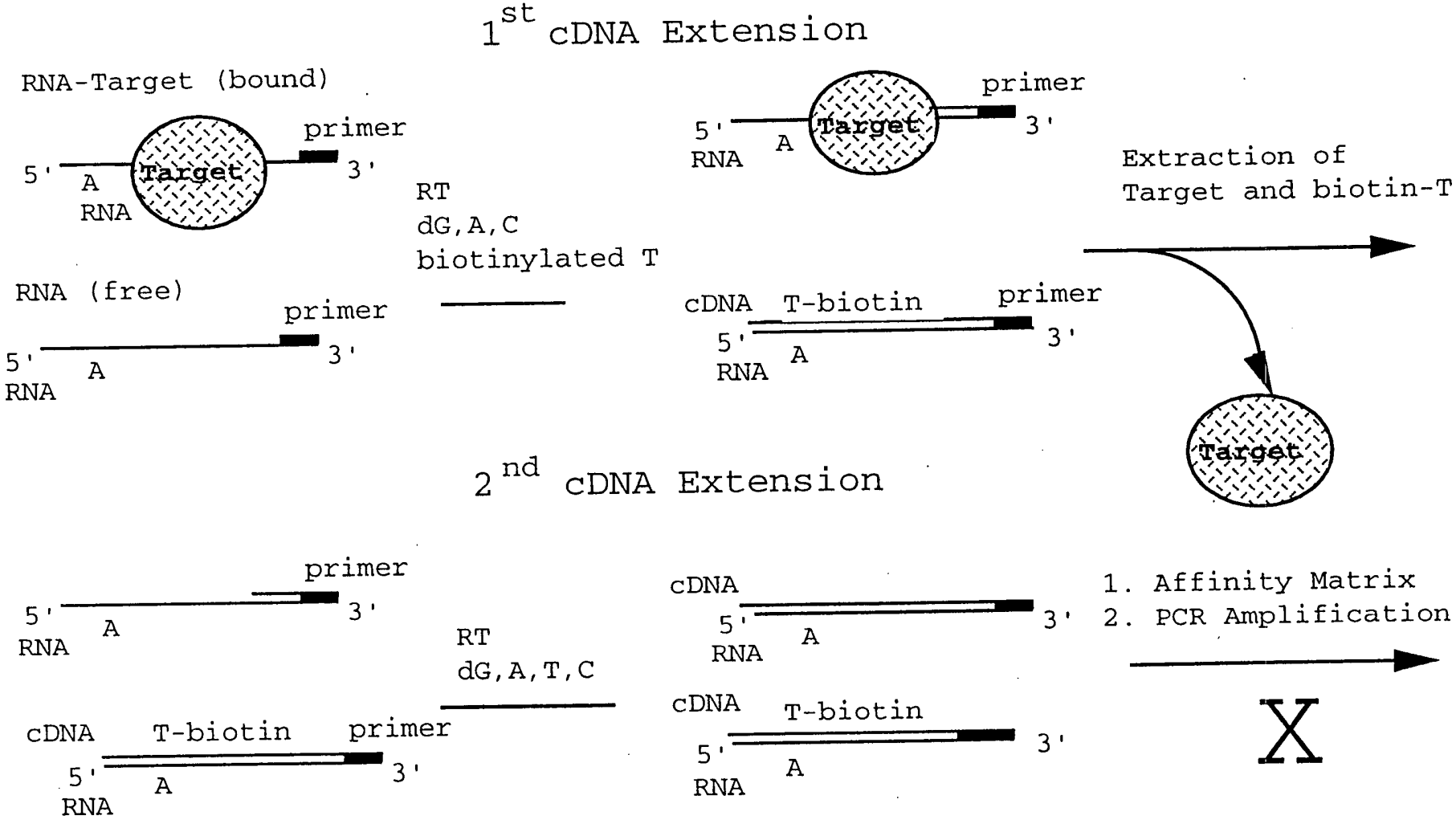

1st cDNA Extension
RNA-Target (bound)
primer
5'
A
Target
3'
RNA
RT
dG,A,C
biotinylated T
RNA (free)
primer
5'
A
3'
RNA
primer
5'
Target
3'
RNA
A
Extraction of
Target and biotin-T
cDNA
T-biotin
primer
5'
A
3'
RNA
Target
2nd cDNA Extension
primer
5'
A
3'
RNA
RT
dG,A,T,C
cDNA
T-biotin
primer
5'
A
3'
RNA
cDNA
5'
A
3'
RNA
1. Affinity Matrix
2. PCR Amplification
cDNA
T-biotin
5'
A
3'
RNA
X

FIG. 25

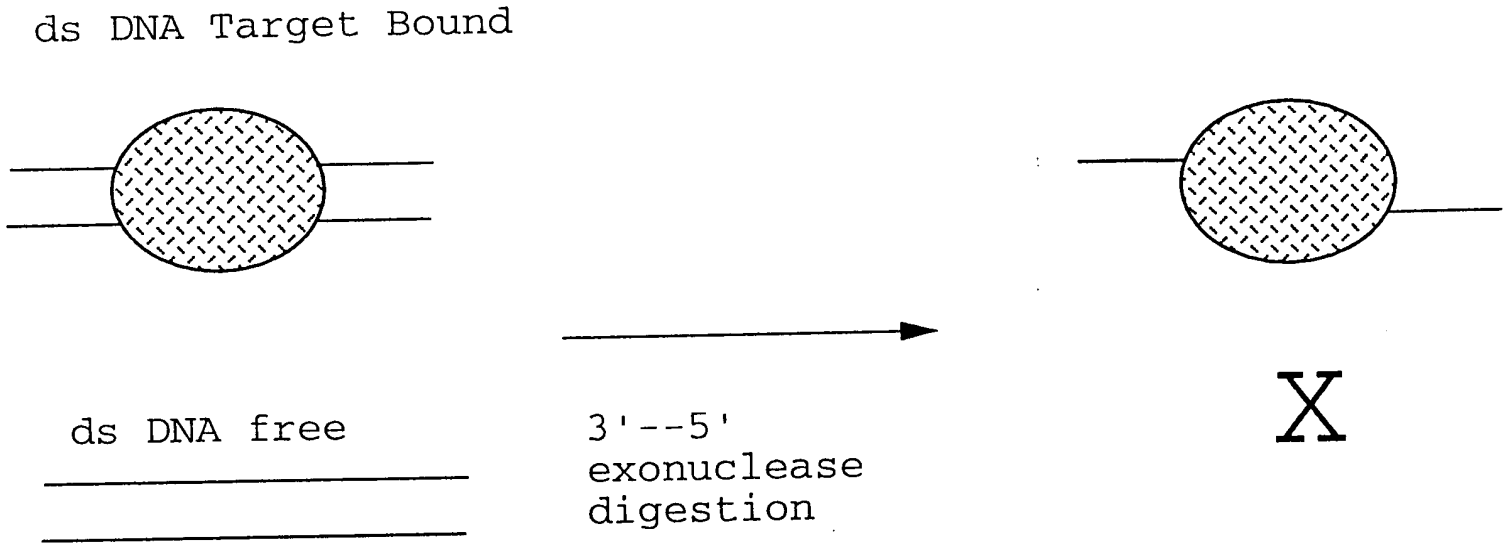

ds DNA Target Bound
ds DNA free
3'--5'
exonuclease
digestion
X
Remove Target
Primer Extension
Regenerated
Double-stranded
High Affinity
Candidates

FIG. 26

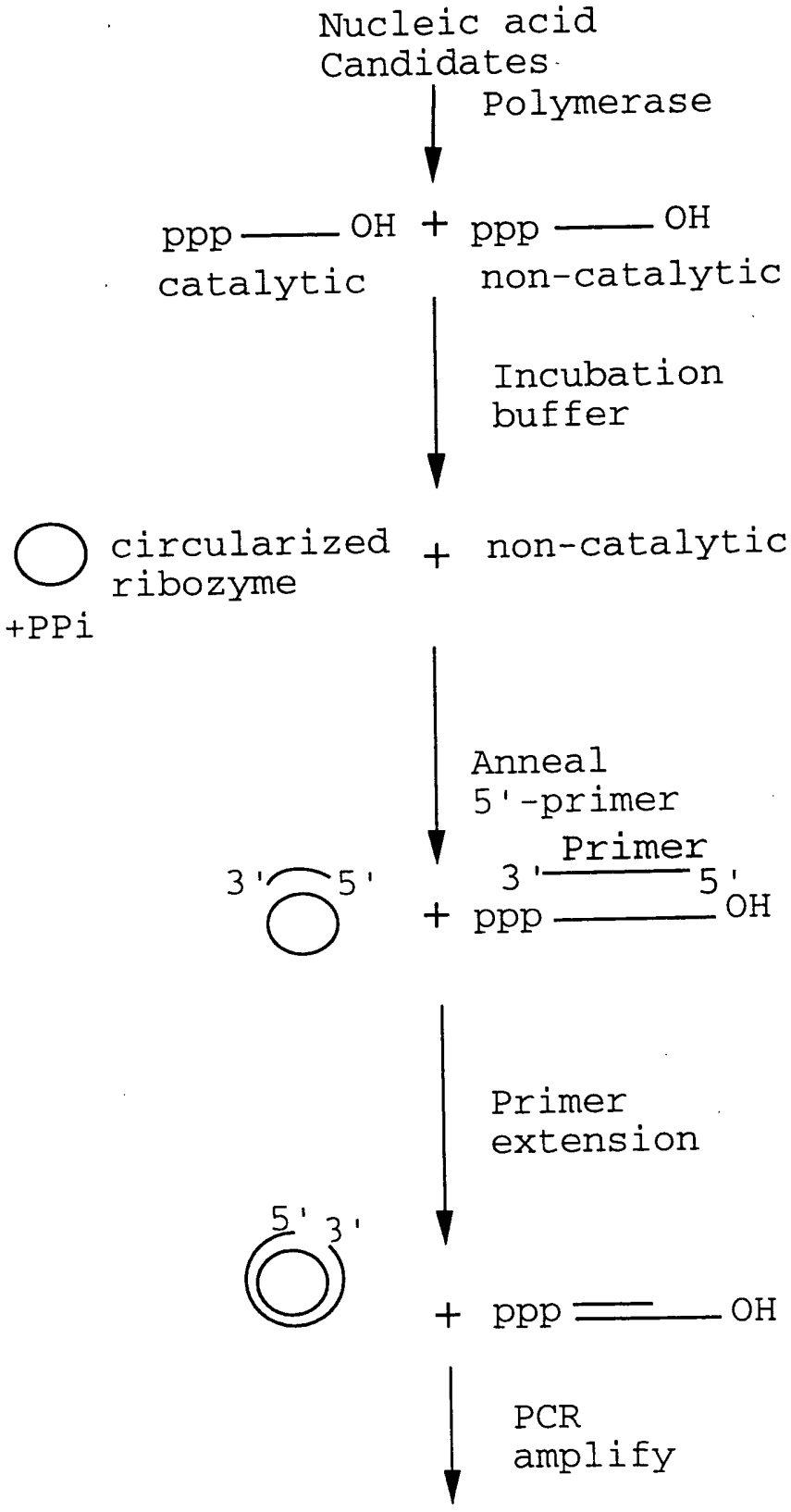

Nucleic acid
Candidates
Polymerase
ppp OH + ppp OH
catalytic
non-catalytic
Incubation
buffer
circularized
ribozyme
+PPi
+ non-catalytic
Anneal
5'-primer
Primer
3' 5'
3' 5'
+ ppp OH
Primer
extension
5' 3'
+ ppp OH
PCR
amplify

FIG. 27